

THE COLOUR CHANGES IN LIZARDS,  
PARTICULARLY IN *PHRYNOSOMA*

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(Received 10 April 1937)

(With Two Plates)

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I. INTRODUCTION

OF all animals chameleons have most excited the attention of students of colour changes. These lizards are limited to the Old World and almost exclusively to Africa. In the New World the only competing forms are the two iguanids *Anolis* and *Phrynosoma*, neither of which are to be compared with the chameleon in variety of colour change or in convenience of size for experimental work. Chameleons are not placed by systematists near the iguanids; consequently it is not to be expected that a detailed agreement will be found between the results of those workers who have investigated chameleons and of those who have worked upon the lizards of the New World. Many differences in the conclusions of these two sets of investigators are to be attributed to this state of affairs and it is to be regretted that no one worker has had the opportunity of studying both classes of materials.

The studies upon which the following paper is based were carried out on one of the common western "horned toads", *Phrynosoma blainvillii* Gray, from the region about Pasadena, California. This lizard hibernates in southern California till early

in March when it emerges to begin its warm-weather activities. I was enabled to study the colour changes in this *Phrynosoma* through an invitation accompanied by a considerable grant from the California Institute of Technology. To the officers of the Institute I wish to extend my sincere thanks for their generosity. To Dr T. H. Morgan, Director of the Kerckhoff Laboratories of the Biological Sciences, where my work was done, and to his very efficient corps of assistants I am under obligations for an ample supply of living horned toads and for excellent laboratory facilities for work.

Many of the investigations herein described called for operative procedure. Whenever this was the case the horned toads were first stupefied by immersion in finely cracked ice for some 10–15 min. Such treatment induced an anaesthesia that lasted much longer than any operation. By this method drugs were avoided, many of which are extremely disturbing to subsequent observations. The final recovery of the lizard after having been chilled is relatively quick and satisfactory.

## II. NORMAL COLOUR CHANGES OF *PHRYNOSOMA*

*Phrynosoma blainvillii* has a well-defined dorsal colour pattern (Pl. I, fig. 1). This consists of two large, lateral, black patches on either side of the neck, four dark bands across the trunk and about five across the tail. All these dark areas are essentially unchangeable; they are separated from each other by pale areas which are subject to very considerable alterations in tint. The pale areas may vary from a dark ashen grey to almost white. In the pale stage the head of the horned toad is almost completely white and a narrow band of a pronounced pale tint extends down the middle of the back. Colour changes are best judged not on the general surface of the body, but on the slightly mottled legs either anterior or posterior and on the row of dentate scales which are some thirty in number and range along the edge of each side of the trunk from the anterior leg to the posterior (Pl. I, figs. 1, 3 and 4). These scales change with great precision from a creamy white (Pl. I, fig. 3) to a tint in which the body of the scale is nearly black (Pl. I, fig. 4). The changes in tone of those parts of *Phrynosoma* that are capable of change are dependent for this activity upon their melanophores which by concentrating or dispersing their pigment blanch or darken the animal. This has been demonstrated on various lizards again and again from the days of Brucke (1852) to recent times (Schmidt, 1918). There is not the least evidence that these colour changes are accomplished by the rapid destruction of pigment and its reformation as claimed by Ruth & Gibson (1917) for certain Philippine lizards. Beside melanophores the skin of *P. blainvillii* contains xanthophores and in consequence the paler parts of this lizard may often take on a strong yellowish tint. In the subsequent account in this paper attention will be given to the activities of the melanophores exclusively, though the xanthophores are certainly also worthy of serious study.

The times required for lizards to change from pale to dark and the reverse varies more or less as Table I shows. In all recorded instances, however, the change from

pale to dark is accomplished more rapidly than that from dark to pale. My early records of these times for *P. blainvillii* set down in 1906 agree well with the new determinations made at Pasadena except that having a very abundant supply of individuals in the recent work I found a greater degree of variation than in my earlier studies. In this lizard the main extent of the change from pale to dark is made in about a quarter of an hour though the process can still drag out very slowly for a number of hours or even days. The opposite change also exhibits a rapid phase of half an hour or so after which it continues slowly for a day or more. In fact both these changes may continue very slowly over a considerable time, for it is impossible to recognize an unquestionable end-point to them. Among the lizards at my disposal a few were found that when placed in either black-walled, illuminated boxes or similar white-walled ones failed in the course of even some days to show any change of tint. This was especially true of certain dark individuals. All these resistant lizards, however, changed pale when injected with adrenalin or dark when injected with pituitrin so that they were thus shown to be capable under such circumstances of changing colour. In this respect *Phrynosoma* is like the spotted frog, *Rana pipiens*, in which the illuminated environment is not an invariable means of inducing alterations in tint and very unlike the killifish, *Fundulus heteroclitus*, which changes with the environment pale or dark with great certainty and precision.

Table I. *Times for changes in various species of lizards from pale to dark and the reverse as recorded by different authorities*

Species of lizard	Authority	Pale to dark	Dark to pale
<i>Chamaeleo vulgaris</i>	Brucke, 1852	Few min	$\frac{1}{2}$ hr
<i>C. pumilus</i>	Zoond and Eyre, 1934	4 min	Slow
<i>Anolis carolinensis</i>	Carlton, 1903	4 min	25 min.
<i>A. carolinensis</i>	Kleinholz, 1935	5-15 min.	10-30 min.
<i>A. equestris</i>	Hadley, 1928	1 min.	25 min
<i>A. porcatus</i>	Hadley, 1928	1 min.	15-18 min.
<i>Phrynosoma blainvillii</i>	Parker, 1906	15 min.	Over $\frac{1}{2}$ hr.

When *Phrynosoma blainvillii* is blinded by the excision of its eyes, it ceases to respond to a white or a black illuminated environment by appropriate changes in tint, but it is not without characteristic colour changes. After enucleation this lizard assumes a tint between pale and dark and rather toward the dark phase than the pale one. When such individuals are placed in a dark room they blanch slightly and when they are put under bright light they darken somewhat. Although they have a well-developed pineal eye (Ritter, 1891) obvious from the exterior they never showed responses that indicated that this organ had any influence on their colour changes. Of four blinded horned toads two were put in a dark room and two under bright light. After  $6\frac{1}{2}$  hours those in the dark room had become noticeably pale and those in bright light dark. These tints were maintained under similar environments for some 5 hours longer. On interchanging the two groups the dark lizards in an hour and a half became pale in darkness and the pale ones dark in bright light. After these preliminary tests the pineal eyes of two of the four lizards were covered with

an opaque black paint and all four were put in the dark. They all blanched moderately and equally in the course of 3 hours and on being transferred to bright light they darkened moderately and in about the same time. In these reactions I was unable to distinguish any difference between the lizards with exposed pineal eyes and those with covered ones. Thus I found nothing in *Phrynosoma* to support the opinion advanced by Clausen & Mofshin (1936) concerning *Anolis* that its pineal eye is a photoreceptor.

Although from time to time I saw evidence of what I took to be a daily rhythm in the colour changes of *Phrynosoma blainvillii*, such as was noted in *P. cornutum* by Redfield (1918) and in *Chamaeleo pumilus* by Zoond & Eyre (1934), I did not follow these interesting reactions further than to see that they were not introducing discrepancies in my own work. So far as I could judge, this type of reaction was not so pronounced in the species on which I worked as it was in Redfield's material. This, however, may have been due to seasonal differences, for all my work was concentrated in the spring of the year, whereas Redfield's investigations extended over several seasons.

### III. NERVOUS CONTROL

It has been almost universally conceded by those who have worked on lizards that these reptiles control their colour changes through nerves (Brücke, 1852; Bert, 1875; Keller, 1895; Carlton, 1903; Parker, 1906; Redfield, 1918; Hogben & Mirvish, 1928*a*, 1928*b*; Zoond & Eyre, 1934; Zoond & Bokenham, 1935; Sand, 1935; Hogben, 1936). In *Phrynosoma* this was first experimentally demonstrated by Redfield (1918) who showed that when the nerve to the hind leg of this lizard was stimulated with a weak faradic current, the leg blanched in that there was a concentration of pigment in its melanophores. As the following account will make clear, this observation has been abundantly confirmed in my own tests.

If a faradic current is applied by platinum electrodes to the roof of the mouth of an anchored, quiet horned toad, the animal will quickly and maximally blanch. When the application of the current, which should be strong enough to tingle slightly the dampened human finger, is continued 1 min., the lizard will begin to blanch in about that time, reach a complete paleness in some 10 min. after which it will begin to darken again. In from half to a quarter of an hour after the first application of the current the animal will have returned to its original dark tint. This test was many times repeated and with remarkably uniform results. In this respect my work confirms the earlier observations of Redfield on *Phrynosoma* (1918) and of Hogben & Mirvish (1928*a*, 1928*b*) on the chameleon. Since these results may be obtained by applying the current to the cloacal wall, as was noted by Redfield, or to the floor of the mouth, they are not to be ascribed to a secondary stimulation of some part of the brain as suggested by Sand (1935, p. 366). When the current is applied to the roof of a lizard's mouth such a stimulation of the brain might indeed be possible, but thus far no experimental evidence has been brought forward to show that this is the case.

When the spinal cord of *Phrynosoma* is severed near the middle of the trunk, the portion of the body anterior to the cut becomes slightly paler than that posterior to it which remains relatively dark (Redfield, 1918). If, now, in such a preparation the floor of the mouth is stimulated electrically, the whole front portion of the lizard from its extreme anterior end to the cut, will blanch very fully in about 1 min. The lizard will then remain in this state for nearly a quarter of an hour after which the blanched portion will slowly darken. The contrast between the pale anterior portion and the dark posterior part is seen clearly in the trunk of the animal, but is most conspicuous on the legs. The anterior pair are very pale and stand out in strong contrast with the posterior ones which are conspicuously dark.

The blanching of the posterior portion of a *Phrynosoma* with severed cord on the stimulation of the cloaca was not always successful. Probably the severance of the spinal cord near the middle of its length disrupts the connexions between the cloacal receptors and the melanophores in a way unlike what happens to the anterior part of the system from the same cut. In this respect *P. blainvillii* is quite unlike the chameleon for it fails completely to show the beautifully graded and segmented relation of cord and melanophore skin areas as has been observed by Hogben & Mirvish (1928*a*, 1928*b*) in the South African *Chamaeleo pumilus*. The spinal cord of *Phrynosoma* must be less highly organized locally than that of the chameleon.

When the floor of the mouth of a dark *Phrynosoma* is electrically stimulated, the whole animal, as already remarked, blanches. The paleness thus produced extends in a very few minutes over all the changeable parts of the lizard. This alteration in tint is to be seen not only in the legs but also over the trunk from its mid-dorsal line to the dentate scales on its extreme lateral edges. The change is generally assumed to be due to the action of concentrating nerve-fibres which accompany the spinal nerves and eventually reach the melanophores. Yet when a single spinal nerve in the midtrunk region of a live *Phrynosoma* is exposed, cut, and even stimulated electrically no band of paleness develops over its area of distribution. This condition has already been recorded by Redfield (1918) and has been repeatedly seen by myself. It has been pointed out by Sand (1935, p. 365) as a contradiction in Redfield's work. It is, however, contradictory only when superficially considered, for, as Zoond & Eyre (1934) have indicated in discussing the dark phase of the chameleon, when only a single spinal nerve is cut no dark band develops. This absence of local darkening is due to the fact that the area of distribution of a single spinal nerve is so fully overlapped by branches from adjacent spinal nerves that the cutting of one nerve does not leave this area really denervated. I have shown to my own satisfaction that when in *Phrynosoma* three or four spinal nerves one next the other are exposed, passed over long platinum electrodes, and simultaneously stimulated, a pale area on the skin distal to the nerves will develop. Redfield's observations, therefore, are not contradictory but illustrate a peculiarity in nerve distribution as clearly pointed out by Zoond & Eyre.

Another approach to the problem of the nervous excitation of the pale phase in *Phrynosoma* is seen in the responses of the lateral border of the trunk and its dentate scales. The total number of these scales on the edge of the body in *P. blainvillii* is

approximately thirty. If a vertical cut is made completely through the body wall of this lizard, parallel with the side of its trunk and from the region of the tenth scale to that of the twentieth, the eight or ten spinal nerves of this region will be transected (Pl. II, fig. 7 *A*). Such a cut may be closed with two or three stitches whereupon it will heal in a few days. If sensitivity tests are made on a lizard so prepared, it will be found that the animal will respond very quickly to a needle prick on the median side of such a cut, but will not react at all to a prick lateral to the cut. Thus it is fair to assume that the region between the cut and the edge of the animal including some ten of the marginal scales is denervated, whereas the area on the median side of the cut is still normally innervated.

Of six lizards prepared as described one soon died, presumably from haemorrhage. The other five, having been allowed 4 days in which to heal their wounds and to recover, were tested in the following way. All the lizards were rendered moderately dark by a sojourn of a day or more on a black background. When in this state each one was stimulated on the floor of the mouth for 1 min. by a faradic current after which they were watched for a change of colour. All five within a few minutes after stimulation blanched freely over the changeable parts of their dorsal surfaces except that portion which was between the cut and the lateral edge including the marginal scales. This area remained dark, and as far as could be judged showed no change in colour whatsoever. Thus in these instances the general blanching occurred over the innervated surfaces, but was absent from the denervated areas, a condition indicative of the presence of concentrating nerve-fibres.

After these animals had returned to their original dark states, which happened in some 20–25 min., two of them were injected with 0.5 c.c. adrenalin (Parke, Davis and Co.), whereupon they again blanched, this time completely including the melanophores of the denervated area; thus it was shown that the colour cells of this area had not been incapacitated for this change by the operation.

A third set of tests on the nervous control in the pale change was made on the legs of *Phrynosoma*. By cutting a longitudinal incision through the skin on the right side of the median dorsal line and immediately over the pelvis, the two bony connexions between the vertebral column and the ilium of the given side could be exposed (Pl. II, fig. 7 *B*). By fine bone forceps each of these bony connexions could then be snipped through on the one hand next the column and on the other next the girdle. After the careful removal of these two pieces of bone there could be seen immediately below where they had been the four main nerves of the lumbosacral plexus and the artery to the hindleg. Two of these nerves were anterior and two posterior to the artery. All four nerves could be cut without injury to the artery and thus the dependent leg could be fully denervated. Four lizards after having been prepared in this way were allowed to recover and darken. On stimulating them electrically on the floor of the mouth all four quickly blanched all over except on the denervated right legs which remained dark as the lateral denervated edges of the trunk had done. The contrast between the innervated pale left leg and the denervated dark right one was most striking and afforded a more conspicuous spectacle than the denervated marginal scales of the earlier test did. Similar trials with similar out-

comes were made on the front legs of other lizards. The denervated, dark legs blanched as the marginal scales did when adrenalin was injected into the lizard.

In a final test of this question nerve stimulation was employed. Four lizards were darkened by an injection into each of 0.1 c.c. obstetrical pituitrin. While in this state each lizard was decapitated, stretched out on its back, the body cavity opened by a cut through the body-wall, and the femoral nerves to the hindlegs dissected free. Each nerve was cut near its origin from the lumbosacral plexus. That to the right leg was passed over the platinum electrodes from an induction apparatus, and with both legs darkened the right nerve was stimulated for 3 min. with a faradic current. In from 5 to 6 min. the leg whose nerve had been stimulated was well blanched from the knee down to the foot. All four specimens showed this reaction well. Their opposite legs with nerves cut but unstimulated remained dark (Pl. I, fig. 2). The left legs of two of the four lizards just mentioned were then tested further. Each leg to begin with was in the dark phase. Its femoral nerve, severed from the spinal cord, was cut again about opposite the middle of the femur and the central end of the stretch of nerve thus isolated was stimulated electrically. In this instance there was no blanching of the leg below the knee as in the former case, showing that this blanching was dependent upon nervous connexion with the distal part of the femoral nerve. When the peripheral end of the nerve at the new cut was stimulated the lower leg blanched fully.

Since general blanching may be excited reflexly by faradic stimulation of the mouth and local blanching by stimulating nerve trunks, and since both these types of blanching may be locally blocked by nerve cutting, I conclude that *Phrynosoma* possesses concentrating melanophore nerve fibres which are directly concerned with its pale state.

I have found in *P. blainvillii* no evidence whatever of dispersing nerve fibres. In no instance in the hundreds of operations in which nerves have been cut in this lizard has the resulting denervated region shown any tendency to darken. This is in agreement with Redfield's results (1918) on *P. cornutum*, but in strong contrast with what is known of the chameleon. In this lizard the severance of a nerve of any considerable size or of a group of nerves has been invariably followed by a darkening of the skin denervated thereby (Brücke, 1852; Bert, 1875; Keller, 1895; Hogben & Mirvish, 1928*a*, 1928*b*; Zoond & Eyre, 1934; Sand, 1935). In the chameleon these patches remain dark for some 3 weeks to become pale only after 6-8 weeks (Zoond & Eyre, 1934) in all probability as the result of nerve regeneration. Dark areas of this kind are well known to occur in bony fishes and have been interpreted here and in the chameleon as due to simple nerve paralysis (Brücke, 1852; Pouchet, 1876; von Frisch, 1911; Zoond & Eyre, 1934; Sand, 1935) or to paralysis associated with the blocking of central inhibitory influences (Zoond & Eyre, 1934; Sand, 1935). In bony fishes they have been shown by the recutting of nerves and by cold blocks to result from the excessive and protracted stimulation of dispersing nerve fibres (Parker, 1934*a*, 1936*a*) and not to be associated in any way with concentrating fibres as the older explanations imply. If this later interpretation should be found to hold for the chameleon, as the present fragmentary evidence appears to favour, this lizard

would thus be shown to possess dispersing as well as concentrating nerve-fibres and thus to stand in strong contrast with *Phrynosoma* in which only concentrating nerve-fibres are present. From this standpoint *Phrynosoma* would be very like the elasmobranch *Mustelus* (Parker & Porter, 1934) whose mechanism for blanching is nervous, but whose means for darkening is not, and the chameleon would resemble in its nervous equipment such teleosts as *Fundulus* and *Amiurus*, both of which show double innervation of their melanophores.

#### IV. PITUITARY CONTROL

It is a remarkable fact that of those who in recent years have had the opportunity of studying chameleons no one has made a thoroughgoing attempt to test the pituitary glands of these lizards for possible effects on their colour changes. Even Hogben, to whom we owe so much for the study of this gland in relation to chromatic function, accepted negative results on the chameleon after a very inadequate test (1924*a*, p. 267). He injected 0.5 c.c. of 1 per cent "infundin" into each of four chameleons, and having observed after 4 hours no colour changes in these lizards, he concluded that reptilian melanophores do not respond to pituitary extract as do those of amphibians.<sup>1</sup> Had he tried an extract of the chameleon's own pituitary gland instead of a commercial product, his results might have been different. Subsequently (1924*b*, p. 39) he remarked that it is to be hoped that someone will reinvestigate this subject.

In contrast with the negative opinion expressed by Hogben several American workers have found lizards very responsive to pituitary substances. Thus Hadley (1931) observed that when 0.5 c.c. obstetrical pituitrin (Parke, Davis and Co.), even after dilution of one part in nine of Ringer's solution, was injected into each of several *Anolis iodurus*, the animals became extremely dark though the same amount of injected Ringer's solution was without effect. Noble & Bradley (1933) showed that when the hypophysis was removed completely from *Hemidactylus* it became permanently pale. This also happened when only the intermediate lobe of this gland was taken out. If a part of the intermediate lobe of the pituitary gland was left in the lizard, the animal remained dark. Kleinholz (1935), who worked on *Anolis carolinensis*, found that the loss of the pituitary gland in this species was followed by the complete and permanent assumption of the pale phase (green). Such lizards lived for as many as 10 weeks. On injecting 8 units of *Fundulus* pituitary extract into such a pale *Anolis* the lizard became brown in a dozen minutes and remained so 6 hours. These observations, meagre though they are, show that contrary to Hogben's suspicions, the colour changes of reptiles are profoundly influenced by pituitary substances, and that these substances are from the intermediate lobe of the gland.

<sup>1</sup> Hadley (1931, pp. 323, 328) cites and quotes Hogben & Winton (1922) and Hogben & Mirvish (1928*b*) to this effect. I have been unable to verify either of these references. From the quotation given by Hadley on p. 323 of his paper I infer that the correct reference should have been to Hogben (1924*a*, p. 267).



My own experiments on *Phrynosoma* confirm this general conclusion. The removal of the pituitary gland from the horned toad can be done with expedition and ease. In preparation for this operation horned toads were chilled in cracked ice and, after having been stupefied, were fastened back down on a small operating table. A gular incision was made in that a cut was carried through the integument into the cavity of the mouth and around the inside of the lower jaw from the joint on one side to the chin and back to the joint on the other side. In this way the floor of the mouth was freed on all sides except the posterior. The floor with the tongue could then be turned back and held there, thus exposing freely the roof of the mouth. The whole operation occasioned the loss of almost no blood. The mucous membrane on the roof of the mouth was then cut through over the parasphenoid bone and reflected, and a portion of the bone removed by a small set drill 2 mm. in diameter or by picking and springing the bone out with a fine but firm knife point. By means of a previous dissection kept as a guide the exact region for penetrating the bone in order to expose the pituitary gland was easily identified. After the exposure of the ventral surface of the brain the gland could be readily recognized immediately behind the optic chiasma. It was then cut free from the brain and sucked out with a pipette. The last steps of the operation involved more or less haemorrhage and it was found advantageous to remove the blood with small pellets of absorbent cotton. The wound in the base of the brain case was then covered over, the floor of the mouth returned to its place and held in position by three stitches and the animal set aside to recover. Of some two dozen such operations I had in all about a score of successful ones. The losses appeared to be due to haemorrhage; the overflow blood filled the cavity of the mouth, clotted there, and obstructed respiration. Most lizards recovered well and in a few days were fully healed and normally active. As a rule they lived till, after other experimental operations, it was found desirable to kill them. At the end of my work two lizards that had been hypophysectomized by the method here described were still alive and very active. They had survived the operation by some 4-5 weeks.

All successfully hypophysectomized horned toads, about a score in number, changed pale in a short time after the operation and gave every sign of remaining so indefinitely. I hypophysectomized small numbers of these lizards at a time and the history of one lot will suffice to indicate that of the others. After the operations, in which cracked ice had been used, the lizards were damp and cold and quite dark in colour. Five hours later they had revived in the warmth of the laboratory and were slightly blanched. Seventeen hours after the last operation, all were very active and fully pale. They were kept in a continuously illuminated, black-walled box and remained persistently pale during the next 6 days. At this juncture one of these pale lizards was injected with 0.05 c.c. of obstetrical pituitrin (Parke, Davis and Co.). In a quarter of an hour the injected individual had begun to darken as compared with the two pale ones held as checks and in 2 hours it was very dark. Four hours after injection it was still dark, but apparently not so dark as it had been and finally, 6 hours from the time of the injection, it was as pale as its uninjected companions.

As a check on the possible effects of the operation I carried three horned toads through all the steps of exposing the roof of the mouth and of the ventral surface of the brain, but I omitted the removal of the pituitary gland. All three lizards after recovery remained dark and thus gave evidence that the preliminary operative steps were not responsible for the final blanching of the animals. It is the loss of the pituitary gland by *Phrynosoma* that renders this lizard persistently pale, a paleness which, however, may be temporarily overcome by the injection of pituitrin.

When 1 I.U. of obstetrical pituitrin, that is, 0.1 c.c. of the Parke, Davis and Co. preparation, is injected into a pale horned toad, the lizard will darken in about 10 min. and will remain dark for several days after which it will blanch again completely. If 0.1 of a unit is injected, the lizard will remain dark only an hour or so and then blanch. To smaller amounts of pituitrin, 0.01, 0.001 and 0.0001 of a unit and to Ringer's solution, there was no darkening whatever. As the adult lizard weighs only about 45 g. the effective doses are relatively large ones, showing that this animal is not particularly sensitive to this material.

To measure roughly the strength of the pituitary extract from a gland as a means of darkening a horned toad, a single gland was dissected from a normal, dark *Phrynosoma*, ground up in a small mortar with Ringer's solution and the whole of the extract, 0.07 c.c. in all, injected into a pale hypophysectomized horned toad. The recipient began to darken in about 5 min. after the injection, became very dark in some 25 min. and remained so in an illuminated white-walled box for 2½ days, after which it gradually blanched to full paleness.

The blood of dark horned toads was injected into pale hypophysectomized ones to ascertain if it was a carrier of a dispersing agent. Two dark individuals were bled from the caudal artery, the blood defibrinated and injected into the lateral edge of the body of a pale individual. In 7 min. a dark spot began to appear in the region of injection and remained clearly visible for over an hour. By 2 hours it had completely disappeared. A second test of this kind was made which was similar to the first except that the injection was made into the left front leg. This became clearly dark in 11 min. and remained so for nearly an hour after which it blanched. Finally in a third test 0.03 c.c. of defibrinated blood from dark lizards was injected into the right hind leg of a pale individual. The injected leg began to darken in 14 min., was very dark after half an hour and had blanched again in an hour and a half. When the blood of a dark *Phrynosoma* is injected into another dark one no change in tint takes place as Redfield (1918) first demonstrated. I conclude from the tests just described that the blood of a dark horned toad carries in it an active dispersing neurohumour which appears to be produced by the pituitary gland of this animal.

## V. ADRENAL CONTROL

Redfield (1916, 1918) was the first to call attention to the possible role of the adrenal glands in the blanching of lizards. His views, which were based upon a study of *P. cornutum*, were received with much scepticism by Hogben (1924), Hogben & Mirvish (1928*a*, 1928*b*), and especially by Zoond & Eyre (1934) and

Sand (1935), all of whom, however, worked upon chameleons. *Phrynosoma* is an iguanid and as such belongs to a different suborder of lizards from that in which the chameleons are placed. This is a fact, which in a discussion of this kind is not to be overlooked, for it is quite conceivable that *Phrynosoma* and *Chamaeleo* may be so distantly related from a phylogenetic standpoint, if I may use so antiquated an expression, as to be really quite different in their chromatic organization. I certainly do not share the belief of Hogben & Mirvish (1928*b*) that differences of this kind are unlikely to occur among lizards, nor do I care to commit myself to the opinion of Zoond & Eyre (1934) that a general theory of reptilian colour change is necessarily to be striven for. Diversity rather than uniformity may be the rule.

The injection of effective concentrations of adrenalin into lizards has been followed regularly by blanching. This was first shown by Redfield (1918) for *Phrynosoma* and has also been demonstrated by May (1924) and by Hadley (1931) on *Anolis* and by Hogben & Mirvish (1928*a*, 1928*b*) on the chameleon. A general idea of the concentrations necessary for this reaction are given in Table II where are combined some of Hogben & Mirvish's results from the chameleon with mine from *Phrynosoma* (Parke, Davis and Co., adrenalin). From this table it will be seen that all effective concentrations act in about 10 min. and that their effects last longer the stronger the concentration. It is also evident that the chameleon is less responsive to adrenalin than *Phrynosoma*, for the chameleon ceases to respond at 1:800,000 whereas *P. blainvillii* did not fail till 1:10,000,000 was reached. *P. cornutum* upon which Redfield worked, reacted occasionally to 1:100,000,000.

Table II. *Times of beginning and ending of blanching from injections of various dilutions of adrenalin into Chamaeleo pumilus (Hogben & Mirvish, 1928*a*, 1928*b*) and into Phrynosoma blainvillii*

Dilutions of adrenalin	Times after injection for blanching			
	<i>Chamaeleo</i> (1 c.c.)		<i>Phrynosoma</i> (0.5 c.c.)	
	Began	Ended	Began	Ended
1 : 1,000			10 min	24 hr.
1 : 10,000			10 min	8 hr
1 : 25,000	- 15 min	10 + hr		
1 : 50,000	- 15 min.	10 + hr.		
1 : 100,000	- 15 min	10 + hr	10 min	2 hr
1 : 200,000		2 hr.		
1 : 400,000		2 hr.		
1 : 800,000	No reaction			
1 : 1,000,000	No reaction		10 min	15 min
1 : 10,000,000			No reaction	

Not only is *Phrynosoma* blanched by injections of commercial adrenalin, but also by extracts from its own adrenal glands. These glands are very narrow spindle-shaped bodies of a bright orange colour, situated on the median side of the gonads. One of these glands was triturated thoroughly in Ringer's solution and the extract thus obtained, in all about 0.3 c.c., was injected into a very dark *Phrynosoma*. In 9 min. the lizard was completely pale and remained so approximately a day after

which it darkened again. Four tests of this kind were carried out with results essentially like that described. These observations confirm Redfield's statements (1918) on the blanching of *P. cornutum* to extracts of its own adrenal glands.

The blood from pale horned toads was tested for its effect on dark individuals. Two horned toads that had been kept for 3 days on a white illuminated background till they were very pale were bled from the tail and their blood defibrinated. Of the serum thus obtained 0.03 c.c. was injected into the right hindleg of a dark horned toad and the same amount of Ringer's solution was injected into the opposite hindleg. In 7 min. the right leg was pale and this paleness increased during the following 10 min. after which it gradually subsided. The opposite hindleg showed no change in tint at all. A similar test was made by injecting 0.05 c.c. of defibrinated blood from pale lizards into the right lateral trunk wall of a dark one and injecting the same amount of Ringer's solution into the left wall. Here again in about 10 min. a pale area appeared over the region of the injection of the blood and no change was noted where the Ringer's solution had been introduced. When defibrinated blood from a pale lizard is injected into another pale one, there is in the recipient no change of tint local or otherwise. These observations on the transfers of blood in *P. blainvillii* confirm Redfield's observations (1918) that the blood of a pale *P. cornutum* will blanch locally the skin of a dark one.

My attempts at adrenalectomy on the horned toads studied by me were wholly unsuccessful as compared with those of Redfield on *P. cornutum*. This species must be much more hardy than *P. blainvillii*, for I never had an operated animal survive double adrenalectomy by more than a day. The adrenal glands are easily accessible but their removal requires tying off the post cava and the simultaneous excision of the gonads and parts of their ducts an operation of no inconsiderable extent. The dozen or more lizards on which I operated had been stupefied with cold. They came out of the operation dark in colour, always remained flabby and inactive, and finally, after having become somewhat pale in tone, died. In this respect *P. blainvillii* appears to have been much as Hogben & Mirvish (1928*b*) found *Chamaeleo pumilus* to be. I would not have had confidence in the results obtained on any of my adrenalectomized horned toads; they were on the one hand too fresh from the operation and on the other hand too near death. In these matters *Phrynosoma cornutum* is evidently a much more favourable subject than *P. blainvillii*.

From what has been detailed of the colour changes in *Phrynosoma* it appears quite certain that this lizard, like the chameleon, blanches in consequence of the action of concentrating nerves. The fact that dark horned toads will blanch at least locally when blood from pale ones is injected into them and that they will blanch generally and profoundly to an injected extract from the adrenal gland from their own kind is indicative of a second method of becoming pale. Naturally one would suspect this of involving an adrenal hydrohumour.

When the floor of the mouth of a dark *Phrynosoma* is stimulated electrically this lizard, like others, will turn pale. If this test is tried on a moderately dark *Phrynosoma* the nerves to one of whose hindlegs have been cut, the lizard will blanch generally except for the denervated hindleg which will remain dark. Blanching

under these circumstances will take place in about a minute and the recovery of the dark phase will require a quarter of an hour or more. Such blanching is universally conceded to be nervous in character.

If a *Phrynosoma* whose lumbosacral plexus has been cut on one side of its body and whose tint has been made slightly dark by a small injection of obstetrical pituitrin is tied down to a board ventral side up, it will be found to blanch completely in from 10 to 20 min. This kind of blanching can be shown to affect the denervated leg as well as the rest of the body. If the lizard is now freed and placed in an indifferent, illuminated environment, it will darken somewhat in the course of 2 hours. If this type of experiment is again tried but with a dark *Phrynosoma* in one of whose hindlegs the artery has been tied off, the blanching consequent upon holding the lizard upside down will appear on the whole body except the leg with the ligated blood vessel. The times for the colour changes in this instance will be found to be essentially the same as in the preceding one. These two kinds of tests, of which three of the first kind and four of the second were carried through, make it clear that the blanching of a *Phrynosoma* as a result of having been held upside down is dependent upon the openness of the artery and upon some substance carried in the blood.

An important distinction between the blanching produced by nervous action and that resulting from the blood turns on time relations. Nervous blanching appears in about 1 min. and disappears in a little over a quarter of an hour. Blanching from a blood-carried neurohumour appears in 10–20 min. and disappears in some 2 hours. These differences in times support the view that there are two types of blanching in *Phrynosoma*, and while in all probability they ordinarily act together, they can be measurably separated by the means just described. The evidence here presented leads me to conclude that *Phrynosoma* has two means of blanching, nervous and humoral, and though the evidence for the source of the neurohumour in the second type is not complete, it points very strongly to an adrenal one. I therefore agree with Redfield in his conclusion that *Phrynosoma* has concentrating melanophore nerve-fibres and a concentrating hydrohumour, very probably adrenalin.

Redfield (1918) first pointed out that when noxious stimuli are applied to horned toads whereby they are led to struggle in order to free themselves, they regularly turn pale. He found such stimuli in the faradic current as applied to the mucous surfaces of the mouth and in the disturbance that results from holding a horned toad upside down. Similar conditions were recognized by Hogben & Mirvish (1928*a*, 1928*b*) in the chameleon and the resulting paleness in such animals was called by them excitement pallor. Even as early as 1852 Brücke noticed that when chameleons were much disturbed their colour patterns became more pronounced. Strecker (1928) and later Hadley (1931) also recorded that *Anolis* became pale (green) if unduly excited. Sand (1935) stimulated pale chameleons by pinching their feet and thereby inducing them to hiss and attempt to bite. In from 1 to 2 min. thereafter they became dark. Sand objects therefore to the term excitement pallor, but may there not be an excitement darkening as well as an excitement pallor? Such at least seems to be true of fishes (von Frisch, 1912; Abramowitz, 1936). Till this subject is

more fully studied, however, it will be difficult to reach any final conclusion. In *Phrynosoma blainvillii*, according to my experience, vigorous stimulation such as results in inducing the lizard to struggle is always followed by blanching. Because of the quickness of this response, a minute or so after the application of the stimulus, I am inclined to side with Hogben & Mirvish (1928*a*, 1928*b*) in attributing it to the action of concentrating nerves rather than to adrenalin as suggested by Redfield (1918), but I know of no reason why the change should not be quickly initiated by nerves and then sustained by some such neurohumour as adrenalin.

#### VI. MODE OF ACTION OF PITUITRIN AND OF ADRENALIN

Does pituitrin or adrenalin when injected into a *Phrynosoma* act on its melanophores indirectly through nerves or other structures or directly on the colour cells themselves? I have discussed this question elsewhere (Parker, 1934*b*) in connexion with the action of adrenalin on the melanophores of *Fundulus* and I have there pointed out that the answer to this question can be sought for only in the denervated areas of skin on experimental animals. Only after the melanophore nerves in a given area have been cut and allowed to degenerate can the mode of reaction of these colour cells to such hormones as pituitrin or adrenalin be tested with certainty.

To this end I prepared six horned toads by making lateral cuts in their body walls as already described (Pl. II, fig. 7) whereby a relatively broad denervated area of skin was produced reaching from the cut to the marginal scales. These six lizards were then kept in pens 12 days during which time their wounds healed and the peripheral portions of their severed nerves degenerated. In cold-blooded animals this process is well known to be completed at room temperatures in about 10 days. On testing the areas of skin denervated by the operation it was found that they were quite insensitive in that the lizards made no response when these areas were pricked with a needle whereas they were extremely reactive when the normal skin was so treated.

Three of the lizards had been kept in white-walled illuminated pens and had become in consequence of this sojourn very pale. Into each of these lizards 0.1 c.c. of obstetrical pituitrin was injected and they were watched for change of colour. By 10 min. all three were quite dark and the darkening proceeded at the same rate and in the same way on the denervated areas on the edges of their bodies as it did on the normal innervated areas. No distinction could be made out in the reactions of the two areas. Hence I conclude that when pituitrin is injected into a *Phrynosoma* it causes the melanophores to disperse their pigment not by acting on their dispersing nerves, if such there be, and thus on the melanophores, but directly on these colour cells themselves.

Similar tests with adrenalin were carried out on the three remaining horned toads with denervated areas. These three lizards had been kept in an illuminated dark-walled box and were in consequence dark in colour. After injecting them each with 0.2 c.c. adrenalin 1 : 1000, they quickly blanched and as in the test with pituitrin the whole lizard changed colour uniformly, the denervated area with the normal areas. These results were precisely the same as I had formerly obtained with *Fundulus* and

lead to the conclusion that in *Phrynosoma* as in *Fundulus* adrenalin acts on the melanophores directly and not through the concentrating nerves. I, therefore, concluded that in *Phrynosoma* the melanophores are acted on directly by both pituitrin and adrenalin. In my opinion we are here dealing with instances of the direct stimulation of melanophores, a conception which in this particular field has been favoured among others by Redfield (1918) and by Hogben & Mirvish (1928*a*, 1928*b*), but opposed by Zoond & Eyre (1934) and by Sand (1935).

## VII. TEMPERATURE RESPONSES

The contributions on the responses of reptilian melanophores to differences of temperature have been very fully reviewed recently by Smith (1929). Almost all workers agree in the belief that high temperatures are associated with a pale skin. This conclusion has been supported by observation on chameleons (Brücke, 1852; Hogben & Mirvish, 1928*a*, 1928*b*; Zoond & Eyre, 1934), on *Anolis* (Parker & Starratt, 1904; Smith, 1929; Hadley, 1929), on *Phrynosoma* (Parker, 1906; Weese, 1917; Redfield, 1918) and on a variety of other lizards (de Grijs, 1899). My present studies on *P. blainvillii* entirely support this view. Dark horned toads placed in a black-walled glass jar surrounded with hot water so that the temperature of the air in the jar was constant at about 42° C. became pale in a little less than an hour and remained so some 6 hours. Pale horned toads tested in the same way remained pale over lengthy periods. When dark individuals with denervating cuts on the sides of their bodies, made in the way already described, were similarly tested, the innervated side and the denervated side turned pale gradually and together, showing that this response is not necessarily a nervous one. To test its humoral possibilities I used for local application a hot rubber pad on the end of a glass tube (Pl. II, fig. 6). The glass tube 8 mm. in diameter was flared slightly at the end where a very thin, small rubber bag was securely tied. Into the bore of the glass tube a much smaller tube was inserted till it reached the centre of the bag. This small tube was supplied with warm water which thus filled the bag and escaped from it by the larger tube. On running a steady, generous stream of water through the smaller inner tube the rubber bag could be kept at any desired temperature and applied locally to the somewhat irregular surface of a horned toad. The most favourable region for application was on the dentate scales of the lateral edge of the animal. These scales had been prepared by allowing their nerves to degenerate as already described, and when the rubber bag with an outside temperature of about 37° C. was applied to them, those overlapped by the bag began to blanch in 5 min. and were markedly pale in 10 min. The scales anterior and posterior to the region of application remained dark. When a dark *Phrynosoma* was subjected to the same treatment except that the water circulated through the bag had the temperature of the laboratory, about 25° C., no change in tint occurred, showing that the bag itself had no disturbing effect on the lizard. These tests demonstrate that the change induced by heat is strictly local and cannot therefore be attributed to a hormone, such for instance as adrenalin. Thus blanching from heat is neither nervous nor humoral and I conclude therefore that it results

from a direct stimulation of the melanophores themselves by the heat. In this I agree with Redfield (1918) rather than with Sand (1935).

The effects of a low temperature, either general or local, on the colour changes of lizards is not so simple as that of a high one. Cold has been generally reported to induce a darkening of the reptilian skin (Hogben, 1924*b*; von Buddenbrock, 1928; Smith, 1928; Parker, 1930), a view which has been accepted by many recent investigators (Parker & Starratt, 1904, Smith, 1929, Hadley, 1929, 1931, on *Anolis*; Parker, 1906, Redfield, 1918, on *Phrynosoma*; Hogben & Mirvish, 1928*b*, on *Chamaeleo*). Fuchs (1914) has expressed the opinion that low temperature may merely prevent colour change, and Zoond & Eyre (1934) have pointed out that since a chameleon will become pale in a dark, cold refrigerator low temperatures are not to be regarded as real agents in this response.

In my own tests of the effect of cold on the colour changes of *Phrynosoma* eight pale animals were at different times put into a white-walled jar surrounded with ice and with an inside air temperature of about 4° C. Six of these lizards turned slowly dark in the course of half an hour to an hour and remained so for 4–5 hours thereafter. Two failed to change from their original pale tint during the 6 hours of exposure to the cold. Three dark horned toads when exposed to temperatures of 3–6° C. remained continuously dark for more than 4 hours. All animals tested in the cold were extremely sluggish in their movements.

When the small rubber bag, already described, was chilled by running very cold water through it, 1° C. at the inlet and 6° C. at the outlet, and was applied to the pale, denervated, marginal scales of blanched horned toads, the scales directly under the bag darkened perceptibly in the course of 20 min. to half an hour. Those on either side of the bag remained pale. This test was carried out on three lizards and shows that a low temperature will cause pale scales to darken. This is the conclusion that I arrived at in 1906 and these experiments confirm my original view.

In commenting on this subject Zoond & Eyre (1934) remark that they never have known a pale chameleon in a dark room to change dark at a low temperature. Naturally I was interested to try this test on *Phrynosoma*. Three active horned toads were put in a large glass battery jar in a dark room for a day. At the end of that period all were well blanched. The jar was then immersed in a larger vessel filled with cracked ice and was allowed to remain in the dark room for 3 hours. At the end of this period a thermometer in the air at the bottom of the jar registered 4° C. The three horned toads were sluggish and in tint decidedly dark. A new supply of ice was put in the outer vessel and the test continued over another 3 hours. The air temperature at the end of this period was 4.5° C. and all three lizards were sluggish and markedly dark, so far as could be judged, more so than at the completion of the first 3-hour period. At the end of this time the temperature was 4° C. and all three lizards were dark. They were then put in another vessel in the dark room whose air temperature was 23° C. and allowed to remain in darkness a day. On examination at the end of this interval all three were found to have blanched. These results are directly opposed to those tabulated for the chameleon by Zoond & Eyre. In my opinion both sets of observations are probably correct. The difference between



them is due, I believe, to a native difference in the two kinds of lizards, the chameleon and the horned toad. The skin of the chameleon is very responsive to light or its absence; that of the horned toad, as will be shown presently, is by no means so reactive. The horned toad, on the other hand, is easily stimulated by temperature changes, probably more so than the chameleon. Consequently a chameleon in the dark will remain pale irrespective of low temperature, but a horned toad in the dark will respond to the low temperature and turn dark. This, in my opinion, is the occasion of the difference between the observations of Zoond & Eyre, who worked exclusively on chameleons, and those of such investigators as have studied lizards other than the chameleon. From the standpoint here assumed *Phrynosoma* is a favourable lizard for tests with low temperature; *Chamaeleo* is not. When this whole subject is reviewed it seems to me that Zoond & Eyre's declaration that the work thus far done on it is "singularly unconvincing" is wholly unwarranted and reflects merely an inadequate grasp of the situation.

Since the changes in the melanophores of *Phrynosoma* in response to low temperatures take place locally and in denervated regions, they justify the conclusion that cold affects the melanophores directly.

No one can work on *Phrynosoma* with high or low temperatures without being cognizant of a difference in the lizards under these two conditions. With heat they are active and quick in response. With cold they are sluggish and may even fail to respond. In this sense my observations support Fuch's suggestion (1914) that low temperatures slow down or even abolish reactions. My conclusions are opposed to those of Zoond & Eyre that low temperatures are without real effect.

#### VIII. EFFECTS OF LIGHT AND OF DARKNESS

As Sand (1935) remarks "The evidence for different species of *Chamaeleo*, *Anolis*, and *Phrynosoma* all goes to show that the melanophores of reptiles are contracted when the animals are equilibrated to darkness" (Brücke, 1852; Keller, 1895; Carlton, 1903; Parker & Starratt, 1904; Weese, 1917; Redfield, 1918; von Geldern, 1921; Hadley, 1928; Zoond & Eyre, 1934). In this respect *P. blainvillii* is no exception. Pairs of this lizard, selected for agreement in colour from the general stock when placed one in a dark room and the other in bright light, were regularly found after a few hours to disagree in tint in that the individual in the dark room was paler than its mate in the light.

The local effect of light and of darkness in the melanophores of *P. blainvillii* was tested by methods much the same as those used by Redfield (1918). I extended this technique using individuals whose pineal organs were completely covered with an opaque paint, and whose cut nerves on their left sides had been allowed time enough to degenerate as already described. These lizards finally assumed a tint between pale and dark. In complete darkness they became slightly paler than they had been when in bright daylight and they returned to the darker tint when they were transferred from the complete darkness of the dark room to the bright light of the laboratory. Twelve days after these lizards had undergone the preparatory operations they were

tested in the following way. To ascertain whether light exerted a direct effect upon the melanophores of their skin, one of them in an intermediate phase of colour was placed in a wooden box, whose inner cavity fitted so closely to the animal, that it was restrained from moving about and whose lid was perforated by a hole 1.5 cm. in diameter and directly over the row of denervated lateral scales (Pl. II, fig. 5). The box containing the animal thus secured in position was set on a table in a dark room and directly under a 60 W. electric light which was 20 cm. above the aperture in the cover of the box. About 25 min. after the lizard had been thus exposed the illuminated spot on the margin of its body with the dentate scales began to darken and remained dark as long as the test was continued, about  $1\frac{1}{2}$  hours. This kind of test was repeated on three lizards in all, and in every instance the animals showed a local deepening of tint.

To test the effects of darkness a piece of thin zinc folded upon itself was slipped over the denervated left edge of one of the blinded lizards much as a partly open book might be slipped over a flat piece of board (Pl. II, fig. 8). The zinc whose upper fold measured 2 by 3 cm. covered the dorsal surface of the lizard from its mid-dorsal line to its lateral edge and from a region just behind the front leg of the left side almost to the hindleg of that side. The lower fold of the zinc covered in a corresponding way the ventral surface of the animal. The zinc shield was held in place by strings one of which passed round the neck of the lizard and the other round its hindlegs. The zinc piece was fitted snugly to the dorsal surface of the animal so that when the creature was restrained under a bright light (60-watt electric lamp, 20 cm. above the lizard), the uncovered right half of its body was brightly illuminated and the covered denervated left portion was in deep shade, if not complete darkness. After an exposure of this kind for a period of from three-quarters of an hour to an hour the covered denervated area was found to be slightly but certainly paler than the exposed side.

Subsequently I was enabled to check my results on the effects of light and of darkness on horned toads by using two individuals, survivors of an original group of six, from each of which it was attempted to remove the lateral eyes, cover effectively the pineal eye, remove the pituitary gland, and finally make a lateral cut (Fig. 7) so as to produce a peripheral band of denervated skin. Two only of the six lizards survived all these operations, but these two after a week or ten days recovered fully, righted themselves quickly when put on their backs, and in other respects were normally active. When they were placed in the dark box (Fig. 5) and a beam of light was thrown on their denervated left side, that side in course of time appeared slightly darker than the opposite side. When the denervated left side was shielded by the zinc cover (Fig. 8) from a general illumination, the protected side after an interval of some time became somewhat pale compared with the unprotected one. In a second check I selected from the general laboratory stock of blinded and unilaterally denervated lizards three individuals that were in very close agreement in their tints. One of these was put in complete darkness, another under bright electric light, and a third was kept in the diffuse light of the laboratory. After two hours these three were compared. In each of the three the denervated side and the

normal side were in agreement. The lizard that had been in darkness was slightly paler than the one that had been kept in the diffuse light of the laboratory and the lizard in bright light was slightly darker than the one in diffuse light. From these several tests I conclude, in agreement with Redfield (1918), that the melanophores of *Phrynosoma* are directly influenced by light and by darkness and that darkness, like high temperature, tends to concentrate their pigment and bright light, like low temperature, tends to disperse it. The fact that the tests described in this section were carried out on horned toads, the nerves of whose denervated areas had been given time in which to degenerate before the tests were made, precludes the possibility that axon reflexes could have influenced these results.

#### IX. DIRECT STIMULATION OF MELANOPHORES

The direct stimulation of melanophores in lizards has been discussed recently under the caption of independent effectors by Zoond & Eyre (1934) and by Sand (1935). After a review of the older publications on the subject and a brief addition of their own experimental results, these authors conclude that it would be rash to assert that reptilian melanophores can act as independent effectors. An impartial survey of what has been given in the earlier sections of this paper on the melanophore responses in *Phrynosoma* to different temperatures and to light and to darkness leads to a very different conclusion. In *Phrynosoma* denervated areas of skin will blanch locally to darkness and to high temperatures and will darken locally to bright light and to low temperatures. It is impossible to attribute these responses to nerves, for the nerves were allowed to degenerate well in advance of the tests, nor can they be ascribed to hormones carried by the blood for the responses are strictly local. In my opinion they are due to the direct action of changes of temperature and of illumination upon the melanophores themselves. This conclusion is quite contrary to the view expressed by Zoond & Eyre (1934) and by Sand (1935). It agrees, however, not only with the outcome of Redfield's work (1918) on *P. cornutum* but also with that of Hadley and of Smith on various species of *Anolis*. Hadley (1928) showed that pieces of skin taken freshly from *A. equestris* were dark in bright light and pale (green) in the shade and Smith (1929) demonstrated that pieces of fresh skin from the same species were dark at low temperatures and pale (green) at high ones. Subsequently Hadley (1931) confirmed his earlier results with light on skin fragments from three other species of *Anolis*. Thus all these records are in substantial agreement one with another and further are consonant with what has been made out in the colour changes of many other animals, namely, that darkness and high temperatures both tend to blanch and bright light and low temperature to darken their recipients. In commenting on these matters Sand (1935) remarks that in the light of Smith's work Hadley's observations are "unintelligible", a dangerous word to use under these circumstances.

What Zoond & Eyre (1934) regard as "the most complete proof" that melanophores cannot act as independent effectors is a set of carefully conducted tests on an eviscerated chameleon spread out dorsal side up on a wad of cotton soaked in

Ringer's solution. On the right side of this lizard parallel to its long axis and close to its vertebral column a longitudinal cut had been made severing the spinal nerves of that side of the trunk. The left side remained intact. The operation was carried out in a dark room and the lizard in the beginning was maximally pale. Subsequently it was alternately exposed to daylight and to darkness. The right side, whose nerves had been cut, quickly darkened and then remained continuously black irrespective of its exposure to light or to darkness. The left side changed in tint with the changes in the illumination being pale in the darkness and darkish in the light. These conditions led Zoond & Eyre (1934) to state that "the conclusion is unavoidable that the response of the pigment cells of the chameleon to light is dependent upon the integrity of spinal reflex arcs". On removing the sympathetic chain of the intact side of the chameleon that side also rapidly became black.

Zoond & Eyre would doubtless regard the right side of their original chameleon preparation as darkened in consequence of a paralysis due to the cutting of nerves. The opposite side of this preparation with its changes in tint as a result of differences in illumination, they describe as the seat of reflex activity in which light stimulates the nerve terminals in the skin and thus excites nerve impulses, which after passing through the spinal cord, return to the skin by reflex paths to activate there a dispersion of the melanophore pigment. Such an explanation does not seem to me possible, for it fails to accord with some of the more recent advances in what is known of melanophore-nerve physiology. As I have already pointed out, the cutting of melanophore nerves, such as was practised by Zoond & Eyre on the right side of their chameleon, is not in fishes immediately paralytic in its action, but induces a temporary and excessive activity of the dispersing nerve fibres, hence the pronounced darkening. This activation is so extreme that, as is well known in *Fundulus* and in *Amiurus*, very few other means of excitation can compete with it; hence the affected area remains persistently dark. Such dark areas in living chameleons, according to Sand (1935), eventually blanch. If the right side of Zoond & Eyre's chameleon is dark, not because of paralysis but in consequence of excessive nervous stimulation, there is not the least ground to assume that the left side is nervously stimulated at all. On this side, in my opinion, the melanophores react under the direct influence of light or darkness as they have been shown to do in *Phrynosoma*. For this kind of a test *Phrynosoma* is a much more favourable animal than *Chameleo* for, though it has not so low a threshold for these reactions as the chameleon has, it is devoid of dispersing nerve-fibres whose darkening effects obscure in certain respects the results in the chameleon. It must be clear from this discussion that the conclusion set forth by Zoond & Eyre as unavoidable is far from being so and that there is nothing at present known about the melanophore physiology in the chameleon that is inconsistent with the view that its melanophores are open to direct stimulation. Therefore it is not necessary to assume, as Zoond & Eyre (1934), Zoond & Bokenham (1935) and Sand (1935) have done, that in the chameleon the local responses of its melanophores are due to nervous reflexes involving dermal reception. Hence I agree with Brücke (1852), Bert (1875), Keller (1895), Redfield (1918), Hogben & Mirvish (1928*a*, 1928*b*), Smith (1928, 1929), and Hadley (1928, 1931) in

the belief that the melanophores of lizards are open to direct stimulation. In this respect they are independent effectors. How those who oppose the idea of the direct stimulation of melanophores would explain the action of these colour cells in amphibians where no nerves are directly concerned but where neurohumours affect the melanophores immediately seems to me an insuperable difficulty.

#### X. CRITIQUE OF THE NERVE-MELANOPHORE ORGANIZATION IN LIZARDS

Enough information is now at hand to allow a preliminary formulation of the melanophore system in *Phrynosoma*. In accordance with what has been stated in the preceding sections of this paper *Phrynosoma* may blanch to a greater or lesser degree as a result of the action (1) of concentrating nerve fibres, (2) of a concentrating neurohumour, probably adrenalin, (3) of high temperatures, and (4) of darkness. Of these four the last two are relatively insignificant. *Phrynosoma* may darken as a result of the action (1) of a pituitary neurohumour, (2) of low temperatures, and (3) of bright light. Of these the only really effective one is the first. There appear to be no dispersing melanophore nerve-fibres in *Phrynosoma* such as occur in bony fishes like *Fundulus* and *Amiurus*, where, in addition to other factors, dispersing nerve-fibres oppose concentrating ones.

Such in brief is the melanophore system in *Phrynosoma*. That other elements in its organization will be discovered in the future is unquestionable, but progress to such an extent has already been made that the present understanding may be said to be adequate. In fact it may be said to be more than adequate, for this lizard has two very active and different ways of blanching, concentrating nerves and a concentrating neurohumour, when one would seem to be all that is necessary. This was pointed out by Redfield (1918) who called attention to the fact that in *Phrynosoma* "the responses of the melanophores to direct stimulation and to hormones evidently suffice to bring about all ordinary melanophore reactions without the aid of nerves" and then proceeded to show that in addition to hormones and the like there is "the direct action of nerves" and that "either mechanism alone is capable of causing the melanophore pigment to contract". The quotations just given are used by Zoond & Eyre (1934) as evidence that Redfield's conclusions are "extremely contradictory", a criticism which shows that these authors, as well as Sand (1935), failed in reality to grasp Redfield's meaning.

It would be interesting to compare the melanophore system as worked out in *Phrynosoma* with that in *Chamaeleo*, if only more were known about *Chamaeleo*. It is clear from the work of all investigators from Brucke (1852) to Sand (1935) that the melanophores of chameleons are provided with concentrating nerve-fibres. But it is unknown whether the blood of these lizards carries a concentrating neurohumour as the blood of *Phrynosoma* does. That this simple determination should not have been attempted by one of the several recent students of this subject is remarkable. It is clear, as the result of almost all work, from that of Brucke (1852) to that of Hogben & Mirvish (1928*a*, 1928*b*), of Zoond & Eyre (1934) and of Sand (1935), that *Chamaeleo*, like *Phrynosoma*, blanches when subjected to high temperatures and

to darkness. In fact *Chamaeleo* seems very much more responsive in this respect than *Phrynosoma* does. Brücke in 1852 showed that by light and shadow adjustments patterns could be printed on the skin of *Chamaeleo*, a fact confirmed by Zoond & Eyre (1934) and by Zoond & Bokenham (1935). These workers further demonstrated that when a chameleon was placed in a brightly illuminated, white environment, it darkened for 2 min. before it began to blanch, a response that showed that the direct action of light was more immediately effective than its action through the eyes. The initial darkening was absent when the chameleon's body was in a light-proof sack (Zoond & Bokenham, 1935; Sand, 1935).

It is not known whether *Chamaeleo* darkens through the action of a dispersing pituitary neurohumour in its blood as *Phrynosoma* does. Absence of information on this point is the more noteworthy because those who were most active in settling this question in amphibians have subsequently been the most important workers on the chameleon. That the chameleon, like *Phrynosoma* regularly darkens in bright light and usually does so at low temperatures is obvious from earlier work. *Phrynosoma* lacks dispersing nerve-fibres such as are found in many teleosts. Whether this type of nerve-fibre is present or absent in *Chamaeleo* cannot be stated with certainty. In such fishes as have been shown to possess dispersing nerve-fibres, *Fundulus*, *Amiurus*, and the like, the presence of these fibres is indicated by the darkening of their areas of distribution when they are cut (Parker, 1934, 1936*a*, 1936*b*). In the chameleon dark areas due to the severance of nerves have been known since the days of Brücke and have been recently well demonstrated and illustrated by Zoond & Eyre (1934) and by Sand (1935). If the chameleon is like the teleost in this respect, it probably possesses dispersing nerve-fibres. But quite aside from this question of dispersing nerves *Phrynosoma* never darkens to nerve cutting whereas *Chamaeleo* seems always to do so. Thus *Phrynosoma* and *Chamaeleo*, though they agree in many peculiarities of their chromatic systems, show certain well-marked differences. Such differences, however, are not surprising, for as I have elsewhere stated (Parker, 1936*b*) the day has long since passed when one may expect chromatophoral uniformity in any large group of animals. Contrary to the hope expressed by Zoond & Eyre (1934) that a general theory of reptilian colour change will be found eventually, it is becoming more and more evident that chromatophoral systems involve so many diverse elements that uniformity of combination is not to be looked for. The idea that among vertebrates, the fishes, the amphibians, and the reptiles should each for itself exhibit a characteristic, homogeneous, chromatophoral plan, as maintained by some recent writers, is already out of date.

Views concerning the organization of the vertebrate melanophoric system go back to early days. Brücke's experiments in the melanophore system of the chameleon led him to conclude that the colour cells in this lizard were related to their activating nerves as ordinary muscle-fibres are to their motor nerve-fibres. The dark areas that resulted from the cutting of melanophore nerves were believed by him to be due to paralysis, a conclusion to which he came after a consideration of inhibition as a possible means of explanation. He, therefore, declared that the expanded state of melanophores was their resting state and that their contracted

condition was their active one corresponding to the contracted state of an ordinary muscle-fibre. This very reasonable interpretation was accepted by many of Brücke's immediate followers. When Pouchet (1872) discovered that the portion of the nervous outfit concerned with the control of chromatophores was the sympathetic system, it became evident in consequence of the relations of sympathetic nerves to their effectors that chromatophores were more appropriately compared with smooth muscle-fibres than with skeletal muscle. As tonus is an especially common attribute of smooth muscle many of the papers that followed Pouchet's work interpreted the activities of melanophores in terms of central or peripheral tonus, a position which has been maintained with much reason even to the present time (Zoond & Eyre, 1934; Zoond & Bokenham, 1935; Sand, 1935). Thus Brücke's original conception, modified to meet new discoveries, has come down to us as a more or less natural inheritance.

The chief radical departure from this rather conservative scheme is the one proposed by Zoond & Eyre (1934) in their study of the South African chameleon. They there expressed the opinion that when this animal was pale it was so in consequence of a tonic contraction of its melanophores due to the action of autonomic pigmentmotor fibres. The paralysis that in their opinion resulted when these fibres were cut released the melanophores from this tonus, whereupon they expanded and the region of skin concerned became dark. This expansion occurred normally in animals in consequence of the inhibition of the ordinary tonic influences, an inhibition which could be called forth through the illumination of the animal's dermal photoreceptors or through the stimulation of its retina by light from a light-absorbing background. Stimulation of the retinal elements by light from a light-scattering background caused an inhibition of the original inhibition, thus allowing the tonic state to reassert itself. As a result the melanophore pigment became concentrated and the animal blanched. This mode of melanophore action is reaffirmed by Zoond & Bokenham (1935) as well as by Sand in his general account (1935) of the colour responses in reptiles and fishes. It is far from simple; in fact Sand himself describes (1935) certain parts of it, the inhibition of an inhibition, as "theoretically cumbersome".

But this proposed action system is not only cumbersome; it fails to accord with many of the newly acquired facts in the physiology of melanophore organization. Some of these have already been discussed. First of all it has been shown (Parker, 1934) that the dark areas produced in the skins of many fishes by the cutting of nerves are not due to paralysis as has been believed by almost all workers since the time of Brücke, but result from a temporary excessive activation of the dispersing melanophore nerve-fibres in consequence of their injury. This is shown by the fact that after these areas have blanched, as they will in the course of a few days in, for instance, the tail of a pale *Fundulus*, they may be revived by recutting their nerves slightly distal to the region where they were first cut. If the initial dark area was due to paralysis, no such revival would be possible. Such a dark area is, however, easily thus revived. Moreover, if a cold block is placed on a nerve in a dark area, the portion of the area distal to the block will in a short time blanch somewhat showing

that the dark area itself was not produced by being freed from a central inhibition, but resulted from positive impulses originating in the cut. These dark areas then are neither due to paralysis from the cutting of nerves, as has been generally believed, nor do they result from the freeing of melanophores from a central tonus as claimed by Zoond & Eyre (1934) and their followers (Zoond & Bokenham, 1935; Sand, 1935). They are the outcome of the excessive excitation by cutting of dispersing melanophore nerve-fibres (Parker, 1934*a*, 1936*a*, 1936*b*). If the chameleon is like teleosts in this respect, it must possess dispersing nerve-fibres as well as concentrating ones and its melanophores must be doubly innervated. *Phrynosoma* never develops dark areas on the cutting of its melanophore nerves and whatever the interpretation to be placed upon these conditions may be, it is clear that in this respect the horned toad differs from the chameleon.

The consideration of the reptilian melanophore system given in the foregoing pages shows that this system and the corresponding ones in amphibians, fishes, and crustaceans are alike in that they appear to be based on the action of neurohumours. These substances, the products of such nerve-glands as the pituitary and the adrenal and of the nerve terminals of melanophore nerves, are the activators of the colour cells. Such in brief is the neurohumoral hypothesis as applied to melanophores. It has been my effort during the past few years to elucidate this subject by the study of certain animals favourable for this type of investigation. My position in this respect has been recently described by Sand (1935, p. 368), who in discussing double innervation, remarks that the argument supporting this view "forms an integral part of the theoretic basis of Parker's hypothesis of neurohumours". Nothing could be farther from the facts. Neurohumours may serve as activating substances with two sets of nerves as in *Fundulus*, with one set of nerves as in *Mustelus*, or with no nerves as in *Rana*. The number of kinds of nerves present has nothing to do with the neurohumoral hypothesis. That hypothesis as applied to melanophores is concerned with certain substances as possible activators of these colour cells and is in no necessary way coupled with the sources of these substances. I sincerely regret that I should have written my former accounts of this view in so obscure a way as to have been misunderstood in this respect.

I trust that what has been stated in the present paper may remove this obscurity, for such a presentation of neurohumoralism as that given by Sand has never been, I am sure, in the minds of any of the proponents of this hypothesis.

## XI. SUMMARY

The activities of the melanophore system of *Phrynosoma* in comparison with that of *Chamaeleo* may be stated categorically in the following way.

### I. The blanching of *Phrynosoma blainvillii* is due

- (1) to the action of its concentrating nerve-fibres on its melanophores,
- (2) to the action of a hydrohumour, probably adrenalin, on the same cells,
- (3) to the direct response of these cells to darkness, and
- (4) to high temperatures.



II. The blanching of *Chamaeleo* is due to the first, third, and fourth of these factors. Whether the second factor is effective in this lizard is not yet determined.

III. The darkening of *Phrynosoma* is due

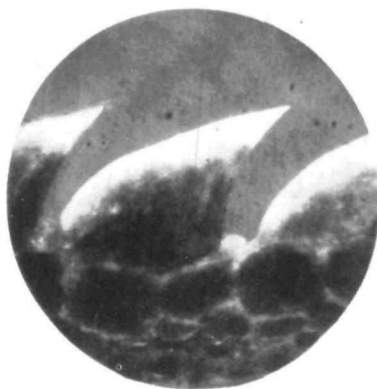
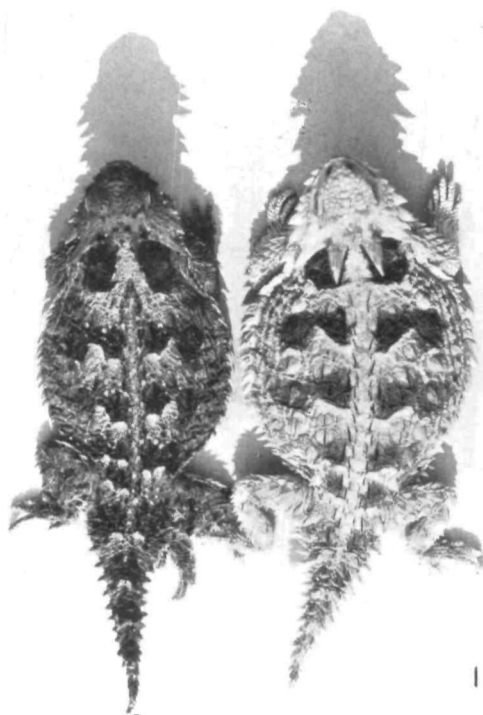
- (5) to the action of a pituitary neurohumour on its melanophores,
- (6) to the direct response of these cells to strong light, and
- (7) usually, to low temperatures.

There are no dispersing nerve-fibres known in *Phrynosoma*.

IV. The darkening of *Chamaeleo* is due to the sixth and probably to the seventh of these factors. This lizard presents strong indications of possessing dispersing nerve-fibres. Whether it darkens from a pituitary neurohumour or not is unknown.

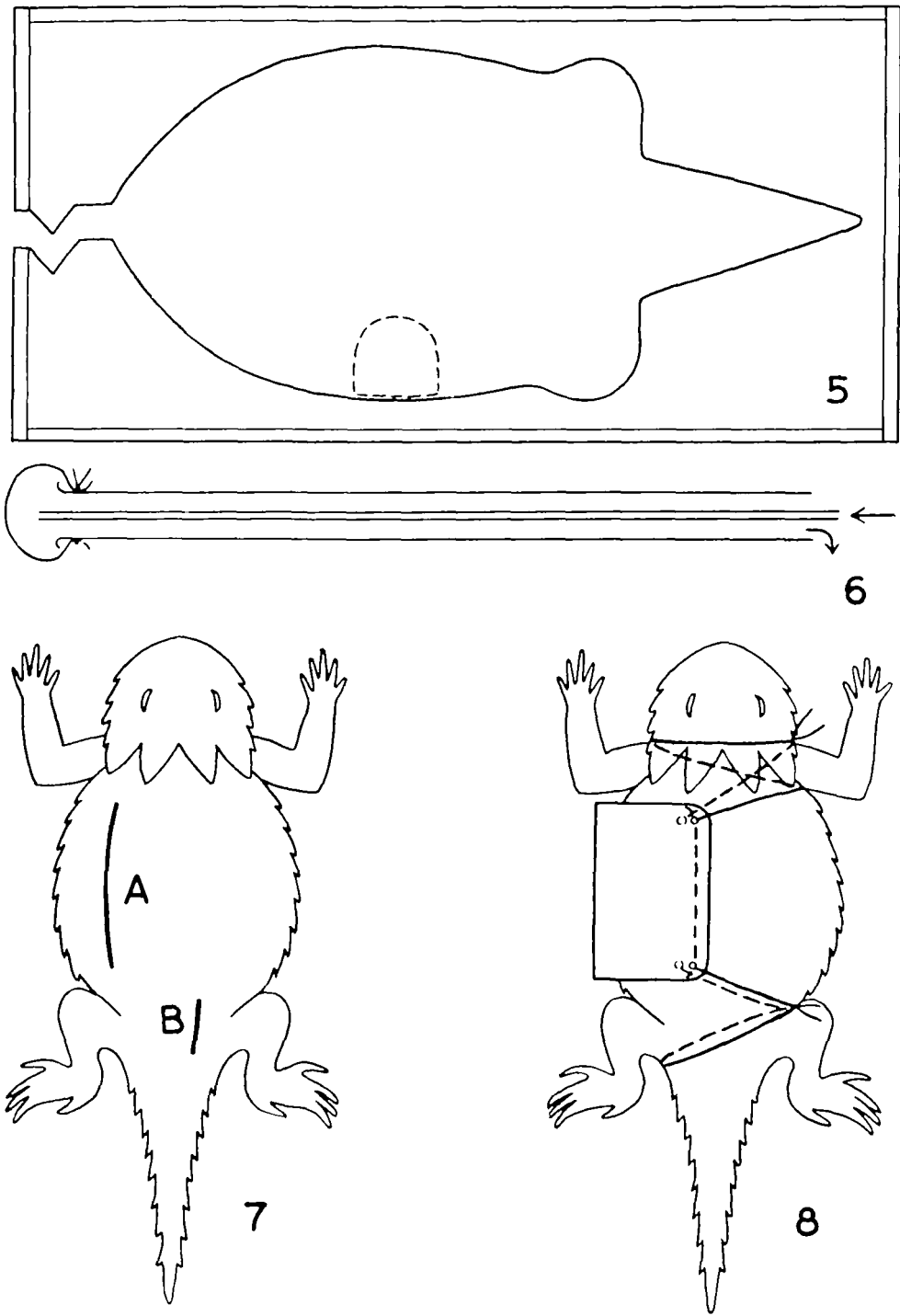
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## EXPLANATION OF PLATES

### PLATE I

For the preparation of the photographs on this plate I am under obligations to Mr G. Keighley and Dr F. M. Carpenter

- Fig. 1. Two horned toads, *Phrynosoma blainvillu*, showing extremes of paleness and of darkness. The one to the right had been some days in a white-walled box under bright illumination; the one to the left in a black-walled similar box.  
 Fig. 2. A dark horned toad, decapitated, with body cavity opened ventrally, and the nerves to both hindlegs cut. The nerve to the right leg was subjected to electrical stimulation for 2 min. with the result that that leg soon blanched; the nerve to the left leg was left unstimulated and the leg remained dark.  
 Fig. 3. Marginal dentate scales from a pale horned toad, enlarged.  
 Fig. 4. Marginal dentate scales from a dark horned toad, enlarged.

### PLATE II

- Fig. 5. Plan of a dark box into the cavity of which (inner outline) a horned toad could be snugly fitted with head to the left in the drawing and tail to the right. At the extreme left is a zigzag opening by which air could reach the unprisoned lizard. When the top of the box was put on, the lizard was in darkness except for a beam of light which could be admitted through a hole in the top whose position is indicated by the dotted outline. This hole allowed the illumination of a spot on the lateral edge of the lizard including some of its marginal, dentate scales.  
 Fig. 6. Local heating or cooling apparatus consisting of a small bag of very thin rubber (left) tied on to a large glass tube through the bore of which a small glass tube led to the cavity of the bag. Water of any desired temperature entered by the small tube, circulated in the bag, and left by the large tube. The rubber bag thus heated or cooled was applied to the surface of the horned toad.  
 Fig. 7. Outline of a horned toad showing the position of the cut (A) for denervating the left lateral edge of the animal with its marginal scales and the cut (B) for exposing its lumbosacral plexus.  
 Fig. 8. Outline of a horned toad showing the attachment of the zinc shield by which the left side of the body was kept in relative darkness when the rest of the lizard was in bright light.